

Fig. 1. pH-Dependence of the hydrolysis of CBZ-ALa-Ile catalyzed by the carboxypeptidase (25°C, 50 mM acetate buffer and 50 mM citric acid/ KH_2PO_4 buffer at pH 2.9 and 3.3)

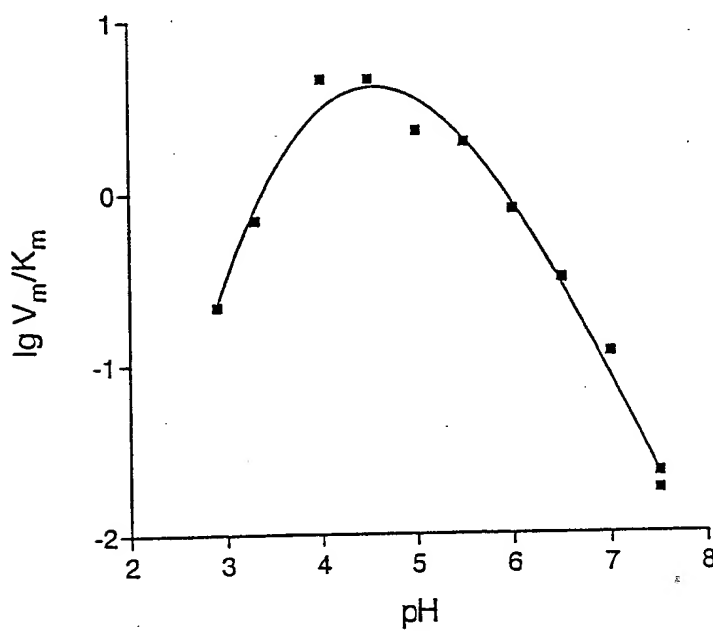
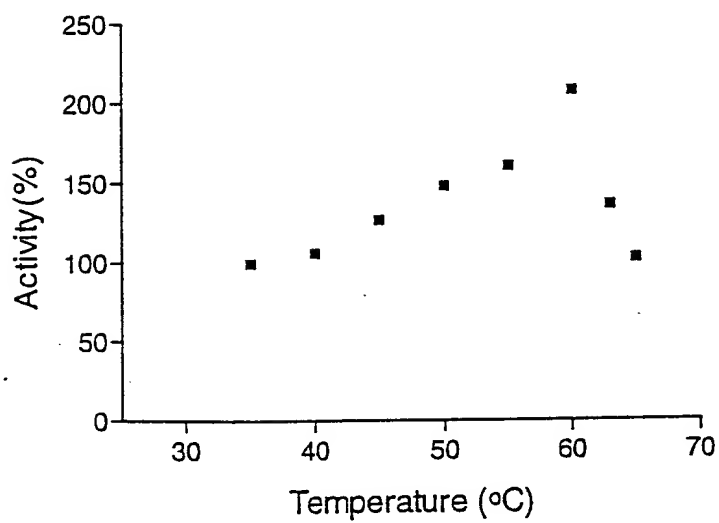


Fig. 2. Temperature optimum of hydrolysis of CBZ-Ala-Ile (1.5 mM) by the carboxypeptidase (50 mM acetic buffer, pH 4.0)



ATCGGTGGCTACGAATTTCTCTCAGTGCTACCCCTTGGTTGACCCAGTTGGCCCTTCCAGGAAGTACACCCGGTCCGTCCGT
 MRGYEFLSVLPLVAASWALTPASVGRRL
 ACCCAAGAACCCACCCGGGTCAAGACTCTTACAACCCGTCACCAATGTCAACCATCCGTACAAGGAACCCGGGACAGAGCCGT
 PKNPTGVKTLTTANNVTIRYKEPGAEGVCE
 CCCCCGGTGTCAATCCTACTCTGATATGTGACACCTCTCCCGAGTCCCATACCTTCTTCTGTTCTTGAAGCCAGACATA
 TPGVKSYSGYVDTSPESTPTFWFFEARNPET
 CCACCTATCACATTGTGGTGAATCGTCCGCTCGAAGCGATTCTTGTATCGGTCTCTTGAAGAGTTGGCCCTTCCATGTC
 APITLWLNGGPGSDSLIGLFEELGPCHVNSTF
 TGATGACTACATCAACCTCACTCGTGAAGCGAGTCTCAATTTACTATTCTGTCCAGCCATTGGAGTCCGCTTTTCATA
 DDYINPHSWNEVSNLLFLSQPLGVGFSSYSDT
 TTGATCGGTCCATTAACTGTAACTCGGTCGTAATTCGAGCTTTCAGGAGTTCAAGCCCGTACCCAACCATGATG
 VDGSINPVTGVVENSSFAGVQGRYPTIDATLI
 GATACTACCAATCTTCCCGACAGAGCCCTTCCGAGATCTTCAAGGATTCTTCTAGTGGACTACCTACCTTGGACTCTACCGTG
 DTTNLAAEAAWEILOQGLSGLPSLDSRVQSKD
 CTTCAGTCTATGACCGAGAGCTATGACCCGCTATGTCCTGCAATCTTCAATCATTTTACGAGCAGAATGAGAGAATTC
 FSLWTESYGGHYGPAFFNHFFYEDNERIANGS
 TTAATCGTGTTCAGTTAATTTCAACTCTCTCGGAATTATTACCCCATCATCGACGAGCCGATCCAGCCCTTACTACCCTG
 VNGVQLNPNLSLGIINGIIDEAIQAPYYPEFAV
 AACAAATACCTACCGTATCAAGCTGTCAACGAGACCGTCTCAACTACATGAAGTTGCCAACAAATGCCAAATGGTTCAG
 NNTYGIKAVNETVYNYMKFANQMPNGCQDLIS
 CACCTGCAACAGACAAACCCACCCGATTACCTGACTACCCCTCTGCCCGAAGCCACCAACATGTCCAGGACATGTTGA
 TCKQTNRTALADYALCAEATNMCRDNVEGPY
 ACCCTTTCTCGTGGTGGTGTGTATGATATTCGGCATCCATATGATGACCCGACTCCGCAAGTTATTACAACAAATTTCTGG
 YAFAGRGVYDIRHPYDDPTPPSYNKFLLAKDS
 GTTCATGACGCTATCGCGTCAACATCAACTACACCCAGTCCAATAATGACGTCTACTACGCTTTCAGCAACAGCGGACTTT
 YMDAIGVNINYTQSNNDVYYAFQQTGDFVWPN
 GTTCATCGAAGACCTCGAGGAGATCCTTGTCTCCCGTGGTGTCTCCCTCATCTATGCCGACCCGATTACATCTGCAACTG
 FIEDLEEILALPVRVSLIYGDADYICNWFGG
 AGCCCGTTTCCCTCGCTCGGAAGTACTCCCAAGCCCGCCAGTTCCGAAGCCGAGGTACACCCCTGAAAGTCAACCGCGTCG
 QAVSLAANYSQAAQFRSAGYTPLKVNGVEYGE
 ACTCCGAGTATGGTAATTTCTCTTCACTCCGCTCTATGAGGACGCCATGAAGTCCCATACTACCAGCCCATCCCTCCCTG
 TREYGNFSFTRVYEAGHEVPYYQPIASLQLFN
 GCGACTATCTTCGGTTGGATATCCAGAGGCCAGAGAAGATCTGCCAGCTACAAGACGAATGGAACGGCTACAGCTAC
 RTIFGW DIAEGQKKIWPSYKTNGTATATHTQ
 CGTCCGTCCCGCTGCTACCGCTACCGCATGTCCAGTGTGGTATG 1668
 SSVPLPTATSMSSVGMA

Figure 3: DNA sequence and deduced amino acid sequence of *Aspergillus oryzae* strain ATCC 20386 carboxypeptidase I. Amino acids determined by protein sequencing of purified *A. oryzae* ATCC 20386 carboxypeptidase I are underlined. Primer positions used in PCR are indicated by dotted arrows. Predicted signal sequence peptide is double underlined.

Consensus

10 20 30 40 50

1 MRGYEFLSVLPVLAASWALPGSTPPASVGRRQLPKNPVTGVTTLTANNVTI
1 - - - - - FVK - - - - -
1 - - - - -
1 MRITSAIASLLLV - - - - - GTATSLQNPHRRAVPAPLTHRSVASRAV
1 MLFRSLLSTAVLAV - - - - - SLCTDNASAAKHGRFGQKARDAMNIANGSANAV
A.oryzae CP1
Penicillium S3
Penicillium S1
A.phoenicis
A.niger

Consensus

60 70 80

51 R - - - - - YK - - - - - EPGAEGVCEET - - - - -
1 - - - - - STKNYRFLNEKTKANLVH - - - - - HL
4 - - - - -
42 - - - - - PVERRSNDFFEYLTNNKTKPYRVE - - - - - SL
48 KHSCLKIPVEDYQFLLNNKTKPYRVE - - - - - SL
A.oryzae CP1
Penicillium S3
Penicillium S1
A.phoenicis
A.niger

Consensus

90 100 110 120

82 - - - - - TFFWFF - - - - -
43 SRALFYI - - - - -
29 - - - - - MW - - - - -
90 SSLF - - - - -
96 SRSLSL - - - - -
A.oryzae CP1
Penicillium S3
Penicillium S1
A.phoenicis
A.niger

Consensus

130 140 150 160 170

129 DYI - - - - -
93 PVE - - - - -
77 PSL - - - - -
140 PVP - - - - -
146 PVE - - - - -
A.oryzae CP1
Penicillium S3
Penicillium S1
A.phoenicis
A.niger

Consensus

180 190 200 210 220

179 QGRYP - - - - -
132 - - - - -
119 - - - - -
179 - - - - -
185 - - - - -
A.oryzae CP1
Penicillium S3
Penicillium S1
A.phoenicis
A.niger

	10	20	30	40	50
1	M R G Y E F L S V L P L V A A S W A L P G S T P A S V G R R Q L P K N P T G V K T L T T A N N V T I				
1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
1	- - - - F V K - - - - -	- - - - -	- - - - -	- - - - -	- - - - -
1	M R I T S A I A S L L L V - - - - G T A T S L Q N P H R R A V P A P L T H R S V A S R A V				
1	M L F R S L L S T A V L A V - - - S L C T D N A S A A K H G R F G Q K A R D A M N I A N G S A N A V				

Consensus

[illegible]

Consensus

	F . . . F W . N G G P G . . S										100										110										120																				
	90																																																		
82	-	-	T	F	F	F	F	F	F	F	E	A	R	H	N	P	E	T	A	P	I	T	L	L	N	G	G	P	G	S	D	S	L	I	G	L	F	E	E	L	G	P	C	H	-	V	N	S	T	F	D
43	S	R	A	L	F	F	F	F	F	F	Q	P	T	I	G	E	P	V	D	E	V	T	I	W	M	N	G	G	P	G	S	S	M	E	S	F	L	Q	E	T	G	R	F	L	W	Q	P	G	T	Y	A
29	-	-	-	M	W	F	F	F	F	F	E	A	R	N	N	P	Q	Q	A	P	L	A	A	W	F	N	G	G	P	G	S	S	M	I	G	L	F	Q	E	N	G	P	C	H	F	V	N	G	D	S	T
90	S	S	L	F	F	F	F	F	F	F	P	S	Q	N	P	D	A	S	D	E	I	T	I	W	L	N	G	G	P	G	S	S	L	D	G	L	L	Q	E	N	G	P	F	L	W	Q	P	G	T	Y	K
96	S	R	S	L	F	F	F	F	F	F	Q	P	T	I	G	E	P	V	D	E	T	T	I	W	L	N	G	G	P	G	S	S	L	E	A	L	S	P	G	E	C	R	F	V	W	Q	P	G	T	Y	Q

Consensus

	N . . N	S W . . . N	. . . N	Q P . G . S	G . S	"	160	170	
	130		140		150				
129	D Y I	N P H	S W	N E V S	N L L F L S	O R	L C V	C F S	Y S D T V D G S I N P V T G V V E N S S F A G V
93	P V E	N P Y	S W	V V L T	N V L W V D	O R	V C T	C Y S	I G T P T A T S Q E E T A - - - - -
77	P S L	N E N	S W	N N Y A	N M I Y I D	O R	V C V	C F S	Y G T - - - - D D V T S T V T A A P Y V - - - - -
140	P V P	N P Y	S W	T N L T	N V V Y I D	O R	A C T	C F S	P G P S T V N D E E D V A - - - - -
146	P V E	N P Y	S W	V N L T	N V L W V D	O R	V C T	C F S	L G V P T A T S E E E I A - - - - -

SECRET

[illegible]

	370	380	390	400	410	Consensus
364	D D P T P P S Y Y N K F L A K D S V M D A I - G V N I N Y T Q S N N D V Y Y A F Q Q T G D F V W P N					A.oryzae CPl
301	S L V Y Q P A G A T V Y F D R A D V K K A L H A P N V T W A E C S N N P V F V G G S S G P E Q E G D					Penicillium S3
270	N D P Y P P K T Y S T Y L S D P T V V K A I - G A R T N Y Q E C P N G P Y N K F A S T G D N P R S -					Penicillium S4
344	- L G F - - - G P D N Y F F N R S D V Q K I L H V P P T D Y S V C S E T V I F A N G D G S - - D P S S					A.phoenicis
353	E V D Y L P A A P A S T L T - A L I K R A M H A P N I T W S E C S V E S V F V G G D G G P E Q E G D					A.niger